

PITHD1 siRNA (h): sc-88421

BACKGROUND

PITHD1 (PITH (C-terminal proteasome-interacting domain of thioredoxin-like) domain containing 1), also known as HT014, TXNL1CL or C1orf128, is a 211 amino acid nuclear protein containing one PITH domain. PITHD1 ectopic expression is thought to promote megakaryocytic differentiation and increase RUNX1 expression, while knockdown of PITHD1 was observed to have the opposite effect on both differentiation and RUNX1 expression. PITHD1 may regulate RUNX1 expression in two distinct fashions, by increasing transcription activity of proximal promoter and enhancing translational activity of an IRES element. Altered gene expression of the PITHD1 gene has been associated with leukemia development, with significant downregulation of expression observed in leukemic samples. The PITHD1 gene maps to chromosome 1p36.11 and is conserved in chimpanzee, rhesus monkey, canine, bovine, mouse, rat, chicken, zebrafish, fruit fly, mosquito, *C. elegans*, *S. pombe*, *M. oryzae*, *N. crassa*, *A. thaliana*, rice, and frog.

REFERENCES

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- Weise, A., et al. 2005. New insights into the evolution of chromosome 1. *Cytogenet. Genome Res.* 108: 217-222.
- Gregory, S.G., et al. 2006. The DNA sequence and biological annotation of human chromosome 1. *Nature* 441: 315-321.
- Abdelmohsen, K., et al. 2009. Ubiquitin-mediated proteolysis of HuR by heat shock. *EMBO J.* 28: 1271-1282.
- de Mateo, S., et al. 2011. Proteomic characterization of the human sperm nucleus. *Proteomics* 11: 2714-2726.
- Lu, B., et al. 2015. Novel function of PITH domain-containing 1 as an activator of internal ribosomal entry site to enhance RUNX1 expression and promote megakaryocyte differentiation. *Cell. Mol. Life Sci.* 72: 821-832.

CHROMOSOMAL LOCATION

Genetic locus: PITHD1 (human) mapping to 1p36.11.

PRODUCT

PITHD1 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PITHD1 shRNA Plasmid (h): sc-88421-SH and PITHD1 shRNA (h) Lentiviral Particles: sc-88421-V as alternate gene silencing products.

For independent verification of PITHD1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-88421A, sc-88421B and sc-88421C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PITHD1 siRNA (h) is recommended for the inhibition of PITHD1 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

PITHD1 (A-10): sc-515392 is recommended as a control antibody for monitoring of PITHD1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PITHD1 gene expression knockdown using RT-PCR Primer: PITHD1 (h)-PR: sc-88421-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.